SURVEY OF GENETIC DIFFERENTIATION IN A COASTAL ZONE INVERTEBRATE: THE ECTOPROCT *SCHIZOPORELLA ERRATA*

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Wise management of shallow marine and estuarine areas—the coastal zone—will require judgments on anticipated changes in the genetics of marine organisms due to the effects of heated waters from power plants, of chemical pollution from solid waste disposal, *etc.* Changes in gene frequencies in local populations will be caused by natural selection acting through these new environmental influences, and such changes will need to be monitored once baseline data for coastal zone species have been established.

A significant result of both laboratory and natural experiments of the past quarter century has been the documentation of the rapidity with which gene frequencies change in local populations. This is well documented in the extensive studies on *Drosophila* (tabulated in Lewontin, 1974). And if this is the case in the fruit fly, then what about other species which, often for reasons of historical accident, are not as well studied?

Data are presented in this paper in order to establish baseline information on certain genetic characteristics of a prominent coastal zone species. Collections are from 14 localities on Cape Cod taken during the summer and early fall of 1972, and repeated in August-early September, 1973. I have used the most abundant and widely distributed marine, subtidal species of docks and pilings—the ectoproct *Schizoporella errata*. *S. errata* is an orange-red encrusting species that builds a calcium carbonate skeleton as it slowly grows out over the substrate. *S. errata* easily overgrows barnacles, worm tubes, and other sessile animals that happened to settle in its path.

S. errata is a useful organism for coastal zone population genetics because of its natural abundance and its coloniality; this would permit laboratory and field experiments using clones grown from particular genotypes. In the Woods Hole region, this species produces larvae from about July 1 into September. Single colonies have clearly delineated borders. When 2 colonies grow into each other, each stops growth and a sharp line forms between them and this is easily observed in the field. A single colony (or genetic individual) is usually an annual event, but some colonies are able to overwinter and to resume growth the following spring. These colonies form the breeding stock early in the season. Larvae can not feed in the plankton and consequently must settle within a few hours. Mean larval transport per generation is probably less than 1 km, and perhaps much less.

Previous studies cited below have established that *Schizoporella errata* routinely outbreeds; that a local, interbreeding population is likely to be over an area no less than on the order of 10×10 m; that no significant selection routinely occurs on exposed *versus* protected sides of pilings correlated with degree of wave action;

TABLE I

Data of ecological interest for localities sampled

Localities	Approximate temperature at warmest season—° C	Sill depth m, low water	Distance to open circulation km	Tidal circulation
(1) Cuttyhunk Channel	20.5	none	open	excellent
(2) Cuttyhunk Harbor	21.5	2.5	0.7	fair–good
(3) Robinsons Hole	20.0	none	open	excellent
(4) Sheep Pen Harbor	22.0	none	open	excellent
(5) Woods Hole	22.5	none	open	excellent
(6) Quissett Harbor	23.5	(2.5)	1.3	fair
(7) Phinneys Harbor (Monument Beach)	23.0	2.0	0.6	fair–good
(8) Green Pond	23.0	2.5	0.8	fair
(9) New Seabury	24.0	1.0	2.0	poor
(10) Lewis Bay (Hyannis)	23.5	3.0	4.2	good
(11) Uncle Roberts Cove	26.5	1.5	4.6	fair
(12) Bass River Inlet	25.0	none	open	good
(13) Stage Harbor	24.0	2.5	1.1	good
(14) Meetinghouse Pond (East Orleans)	27.0	1.5	17.	poor

that genotype and gene frequencies can remain uniform at a given locality for a year; and that gene frequencies at a biallelic leucine amino peptidase locus appears to vary directly as function of environmental temperature measured at the warmest time of the year at 5 localities (Schopf and Gooch, 1971; Gooch and Schopf, 1970, 1971; Schopf, 1973).

MATERIALS AND METHODS

At each of 14 localities on Cape Cod, Massachusetts, and the adjacent islands, I collected by free diving 23 to 83 individual colonies of *Schizoporella errata* in 1972 and again in 1973 from pilings and floating docks. Ecological data (Table I) on temperature, tidal mixing, and distance to the open exchange with the ocean were obtained from observations during collecting, from topographic maps of the U. S. Geological Survey, from coastal charts of the National Oceanographic and Atmospheric Administration, and, most importantly, from discussions with local fishermen and harbor masters.

Slab electrophoresis (Aardvark industries; Lombard, Illinois) using poly-acrylamide was usually performed on 24 individuals per electrophoresis box. Procedures for identifying presumptive gene loci are given in earlier publications (Gooch and Schopf, 1970, 1971). Procedures for staining gels were those reported by Hubby and Lewontin (1966) for leucine amino peptidase (LAP, with the minor change that Fast Black K is added at the same time as the substrate), and by Yang (in Selander, Smith, Yang, Johnson and Gentry, 1971) for glutamate oxalate transaminase (GOT). I will document elsewhere the now fairly extensive data on the degree of genetic variability in *Schizoporella errata* (34 loci, 3 definitely polymorphic, 4 provisionally polymorphic; 9–21% of loci polymorphic) (Schopf, 1974).

Three LAP loci have been reported (Gooch and Schopf, 1970), one of which (LAP-2, hereafter referred to as LAP) is of interest in the present paper. LAP is evidently a monomeric enzyme with homozygotes indicated by a single band (slow or fast mobility on the gel) and heterozygotes indicated by a double band (both the slow and the fast bands). Two GOT loci consistently stain, and of these (GOT-2, hereafter called GOT) is polymorphic and of interest here. GOT appears to be a dimeric enzyme with no activity under normal staining conditions for the slow homozygote. Thus one homozygote (fast) is indicated by a single dark band, and the other homozygote by the absence of a band. Heterozygotes are inferred from a double band which is presumably composed of a lower band (due to one
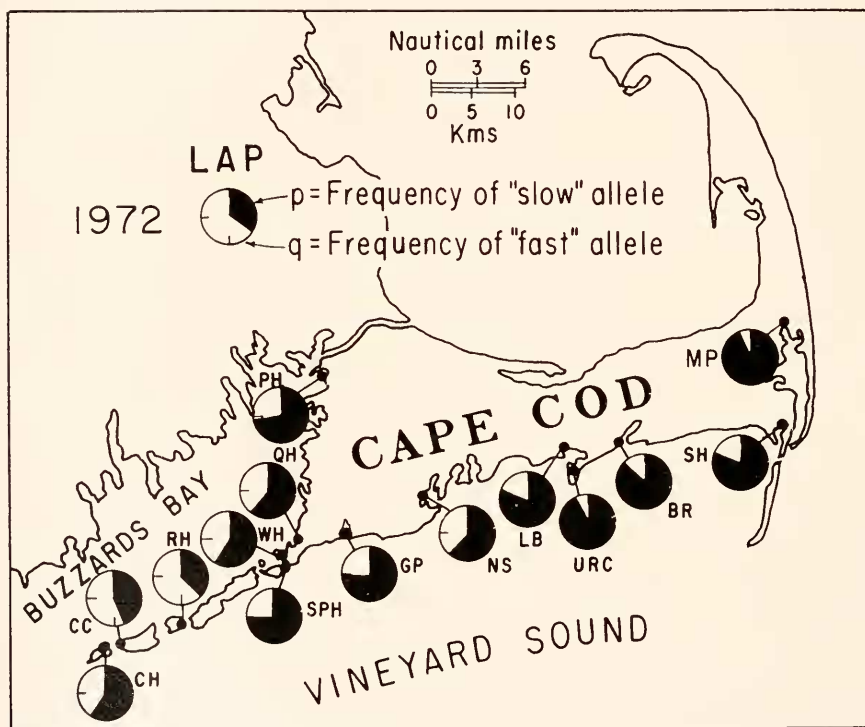

FIGURE 1. Map of allele frequencies (1972) at the polymorphic leucine amino peptidase (LAP) locus. "Fast" and "slow" refer to mobilities of the enzymes on polyacrylamide gels. Note the generally higher frequency of the "fast" allele to the southwest. WH is an average of WH-1 and WH-2. Localities are listed in Table II.

subunit from the fast enzyme) and a middle hybrid band (inferred to be the other subunit from the fast enzyme). The usually expected third band of the heterozygote attributable to a subunit of the slow enzyme gives no activity under experimental conditions. All local populations scored in this manner are within the expectations of the Hardy-Weinberg binomial expansion for a 2 allele model (Schopf, 1974).

Results

Gene and genotype frequencies for LAP and GOT are reported (Tables II and III), and spatial distributions are plotted (Figs. 1–4). For both 1972 and 1973, a progressive change in allele frequencies for both enzyme loci exists throughout the region studied. Allele frequencies at the LAP and GOT loci also vary in a coordinated manner, and both the absolute change and the rate of change are very similar (Fig. 5).

Water temperature at the warmest time of the year (July through early September) is a good predictor of allele frequencies (Fig. 5). For LAP, R^2, the proportion of total variation about the mean allele frequency explained by regression with temperature, is 64.3% (Draper and Smith, 1966, pages 7–26). For the GOT

TABLE II

Genotype and gene frequency for two loci (1972): leucine amino peptidase (LAP) and glutamate oxalate transaminase (GOT). Abbreviations are: n = number of individuals; if two numbers (e.g . 24/25), n for LAP, then n for GOT; slow = slow mobility homozygote; hetero. = heterozygote; fast = fast mobility homozygote; p = frequency of slow allele; and q = frequency of fast allele

Locality		n	Leucine amino peptidase					Glutamate oxalate transaminase				
			slow	hetero	fast	p	q	slow	hetero	fast	p	q
CC	Cuttyhunk Channel	27	5	14	8	0.44	0.56	2	6	19	0.19	0.81
CH	Cuttyhunk Harbor	41	17	15	9	0.60	0.40	9	17	14.5*	0.43	0.57
RH	Robinsons Hole	24/25	2	14	8	0.37	0.63	1	7	17	0.18	0.82
SPH	Sheep Pen Harbor	47/23	27	18	2	0.77	0.23	0	4	19	0.09	0.91
WH-1	MBL VERRILL Dock	59/24	19	33	7	0.60	0.40	4	8	12	0.33	0.67
WH-2	MBL Intake Dock	83/23	34	37	12	0.63	0.37	3	8	12	0.30	0.70
QH	Quissett Harbor	24	10	9	5	0.60	0.40	3	14	7	0.42	0.58
PH	Phinneys Harbor	24	11	12	1	0.71	0.29	2	8	14	0.25	0.75
GP	Green Pond	24	14	8	2	0.75	0.25	9	9	6	0.56	0.44
NS	New Seabury	24	9	12	3	0.63	0.37	6	11	7	0.48	0.52
LB	Lewis Bay	24	16	7	1	0.81	0.19	3	13	8	0.40	0.60
URC	Uncle Roberts Cove	24	20	4	0	0.92	0.08	11	8	5	0.63	0.37
BR	Bass River	23	17	6	0	0.87	0.13	7	12	4	0.56	0.44
SH	Stage Harbor	24	14	10	0	0.79	0.21	6	10	8	0.46	0.54
MP	Meetinghouse Pond	24	20	3	1	0.90	0.10	9	11	4	0.60	0.40

* includes a "fast" allele which occurred in heterozygous state with another (very rare) allele.

locus, 68.3% of the variance in allele frequency is explained by water temperature. For comparison, the correlation with distance for the same localities measured away from Cuttyhunk Channel in the southwest explains 24.8% of the variance in allele frequency in LAP, and 40.2% in GOT.

A purely geogrphic interpretation of changes in allele frequencies does not account for observations at adjacent localities that have the same parent body of water, and differ only in their degree of water circulation and hence temperature. In 3 cases, as the temperature hypothesis predicts, the locality with the more restricted circulation shows a shift in allele frequencies toward a higher representation of "warm-water" alleles. These are (1) Cuttyhunk Channel compared with

Cuttyhunk Harbor (1.5 km distant), (2) Lewis Bay (Hyannis) compared with Uncle Roberts' Cove (2.9 km removed from the open channel), and (3) Stage Harbor (Chatham) compared with its counterpart Meetinghouse Pond (East Orleans), about 22 km "upstream" at the end of a long marine passageway.

Allele frequencies appear to be stable for as long as 5 successive years (Table IV). Data from one year to the next are independent in the sense that at any given locality recruitment by migration and local breeding could form each new population. In fact, some large colonies overwinter and probably would be sampled in successive years. Inclusion of data from overwintering colonies has the effect of damping variations in allele frequencies due to yearly settlement. However
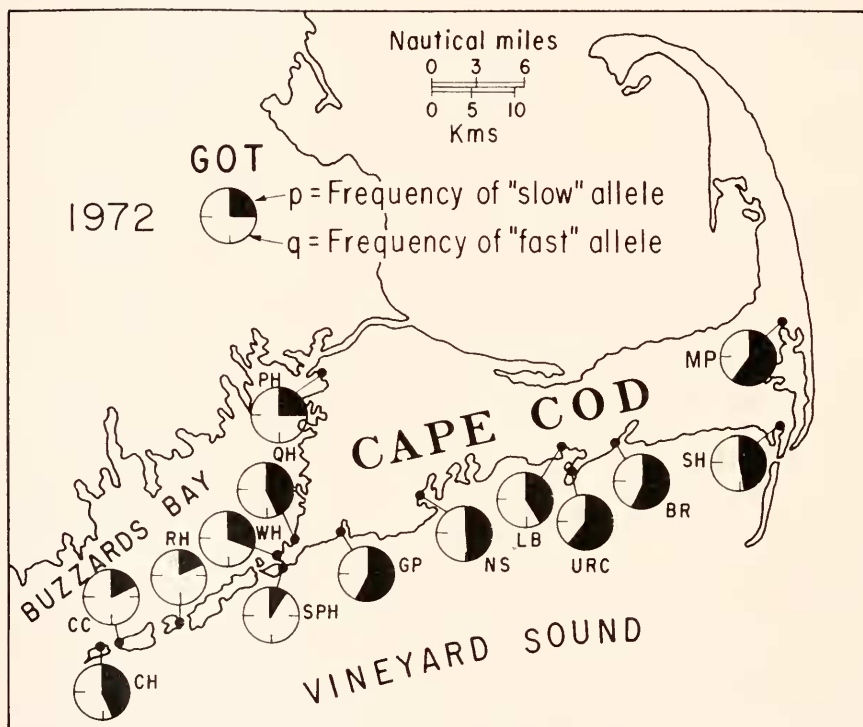

FIGURE 2. Map of allele frequencies (1972) at the polymorphic glutamate oxalate transaminase (GOT) locus. "Fast" and "slow" refer to mobilities of the enzymes on polyacrylamide gels. Note the generally higher frequency of the "fast" allele to the southwest. WH is an average of WH-1 and WH-2. Localities are listed in Table II.

this in no way alters the fact that data on allele frequencies represent the living local population for the year sampled.

Previous studies suggested the possibility of a significant change in allele frequencies with water depth in LAP (Gooch and Schopf, 1971), and this was further investigated. In a test of homogeneity (Dixon and Massey, 1969, page 241) for LAP at the MBL Water Intake Dock, there is a significant difference comparing samples at 0–2 m (p = 0.83) $vs.$ 6–7 m (p = 0.60), ($\chi^2_{(1)} = 6.2$, $P > 0.975$).

However, at the MBL *Verrill* Dock, a significant difference clearly does not exist comparing samples 0–2 m ($p = 0.62$) *vs.* 3–7 m ($p = 0.67$). For GOT, neither dock shows a difference with depth at the 95% confidence level ($\chi^2_{(1)} = 1.81$, $P > 0.75, < 0.90$; $\chi^2_{(1)} = 2.00$, $P > 0.75, < 0.90$).

DISCUSSION

The observed pattern of gene frequencies (Figs. 1–4) conceivably could be due to (1) historical relict, (2) local continuing selection at each generation, or (3) a combination of these. Perhaps local, total selection at each generation comes

TABLE III

Genotype and gene frequency for two loci (1973): leucine amino peptidase (LAP) and glutamate oxalate transaminase (GOT). Abbreviations are; n = numbr of individuals; slow = slow mobility homozygote; hetero. = heterozygote; fast = fast mobility homozygote; p = frequency of slow allele; and q = frequency of fast allele

	Locality	n	Leucine amino peptidase					Glutamate oxalate transaminase				
			slow	hetero	fast	p	q	slow	hetero	fast	p	q
CC	Cuttyhunk Channel	24	8	5	11	0.44	0.56	2	7	15	0.23	0.77
CH	Cuttyhunk Harbor	24	10	14	0	0.71	0.29	5	8	11	0.37	0.63
RH	Robinsons Hole	24	4	6	14	0.29	0.71	2	6	16	0.21	0.79
SPH	Sheep Pen Harbor	24	14	9	1	0.77	0.23	1	3	20	0.10	0.90
WH-1	MBL VERRILL Dock											
	0.5–2.0 m	24	10	10	4	0.62	0.38	2	3	19	0.15	0.85
	3.0–7.0 m	23	9	13	1	0.67	0.33	3	6	14	0.26	0.74
WH-2	MBL Intake Dock											
	0.5–2.0 m	24	16	8	0	0.83	0.17	4	11	9	0.40	0.60
	6.0–7.0 m	24	9	11	4	0.60	0.40	2	6	16	0.21	0.79
QH	Quissett Harbor	24	11	8	5	0.62	0.38	5	8	11	0.38	0.62
PH	Phinneys Harbor	24	13	10	1	0.75	0.25	5	7	12	0.35	0.65
GP	Green Pond	24	14	7	3	0.73	0.27	7	10	7	0.50	0.50
NS	New Seabury	24	11	6	7	0.58	0.42	7	11	6	0.52	0.48
LB	Lewis Bay	24	14	10	0	0.79	0.21	4	5	15	0.27	0.73
URC	Uncle Roberts Cove	24	18	6	0	0.88	0.12	14	8	2	0.75	0.25
BR	Bass River	24	17	7	0	0.85	0.15	10	10	4	0.63	0.37
SH	Stage Harbor	24	11	11	2	0.69	0.31	5	11	8	0.44	0.56
MP	Meetinghouse Pond	24	19	5	0	0.90	0.10	11	7	6	0.60	0.40

closer to accounting for patterns in gene frequencies because of the great ease with which organisms are known to respond to selection pressures (Lewontin, 1974). And the geographic settings in which "warm-water" alleles increase in frequency in more restricted areas have probably existed for only 10 to 100 years (approximately 10 to 100 generations). Presumably the warmer the water becomes over the season, the more favored are the individuals with a compliment of genes that includes the fast, "warm-water" alleles.

For the 14 localities mentioned above, selection is thought to operate during the warmest season because all localities undergo similar low temperatures during the coldest time of the year. In addition, as the regional temperature gradient is re-

versed south of Cape Cod, the "warm-water" allele of LAP is again in high frequency in Delaware, and is fixed at Beaufort, North Carolina (Schopf and Gooch, 1971; Gooch and Schopf, 1971).

I have also obtained LAP and GOT data in 1972 and 1973 for Provincetown, Massachusetts, which is on the north side of Cape Cod and which receives its circulation from the cold water of Cape Cod Bay (summer temperature about 12° C). Three and possibly 4 LAP alleles occur in the Provincetown population, with the allele of highest frequency being of identical mobility as the "cold-water" allele found along southern Cape Cod. However, the water in the Provincetown Harbor where the material was collected is quite warm in the late summer (about 22° C).
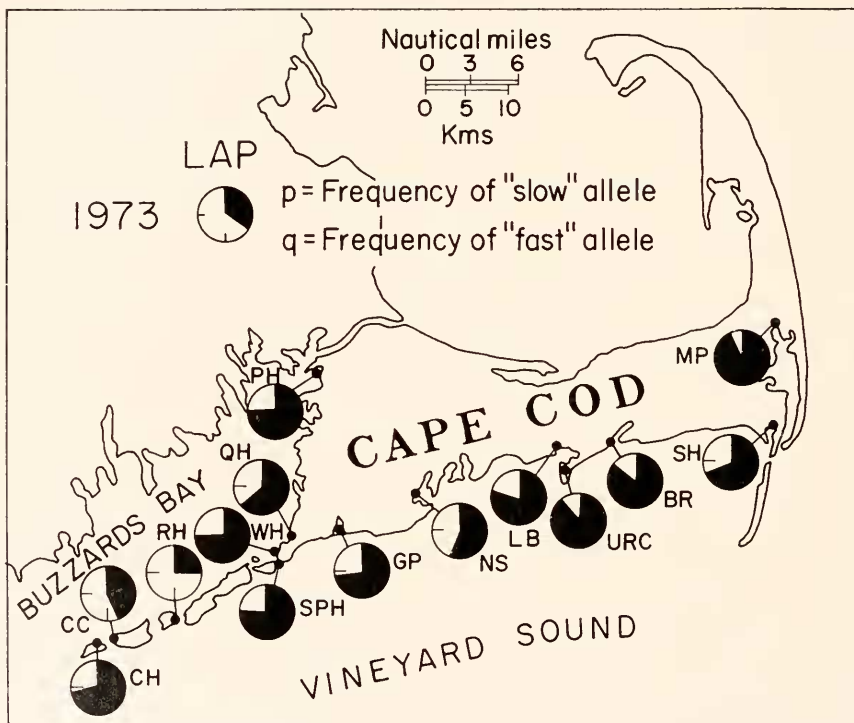

FIGURE 3. Map of allele frequencies (1973) at the polymorphic leucine amino peptidase (LAP) locus. "Fast" and "slow" refer to mobilities of the enzymes on polyacrylamide gels. Note the generally higher frequency of the "fast" allele to the southwest. WH is an average of the WH-1 and WH-2 shallow localities. Localities are listed in Table III.

And in addition the mobility of the allele fixed at the GOT locus is the same as that of the "cold-water" one, both from the transect along the southern shore of the Cape, and, surprisingly, at Beaufort, North Carolina. I have run material from Beaufort, Woods Hole, and Provincetown on the same gel to establish this fact. An additional complicating factor is that the GOT "warm-water" allele is represented in homozygous state on southern Cape Cod by the null activity (as

discussed in Materials and Methods). Biochemical data on these enzyme products would perhaps resolve this issue. From an ecologic point of view, localities in different faunal provinces, as at Provincetown, are probably best compared with places of similar currents, food sources, and water history since the mechanism whereby selection would be acting to cause the observed gene frequencies has not been demonstrated biochemically.

Leucine amino peptidase cleaves N-terminal residues from proteins. Glutamate oxalate transaminase catalyses the reaction of α-ketoglutaric acid + aspartic acid $\rightleftharpoons$ glutamic acid + oxaloacetic acid, and acts to bring aspartic acid into the tricarboxylic acid cycle (Lehninger, 1970). No kinetic data exist on the activity of
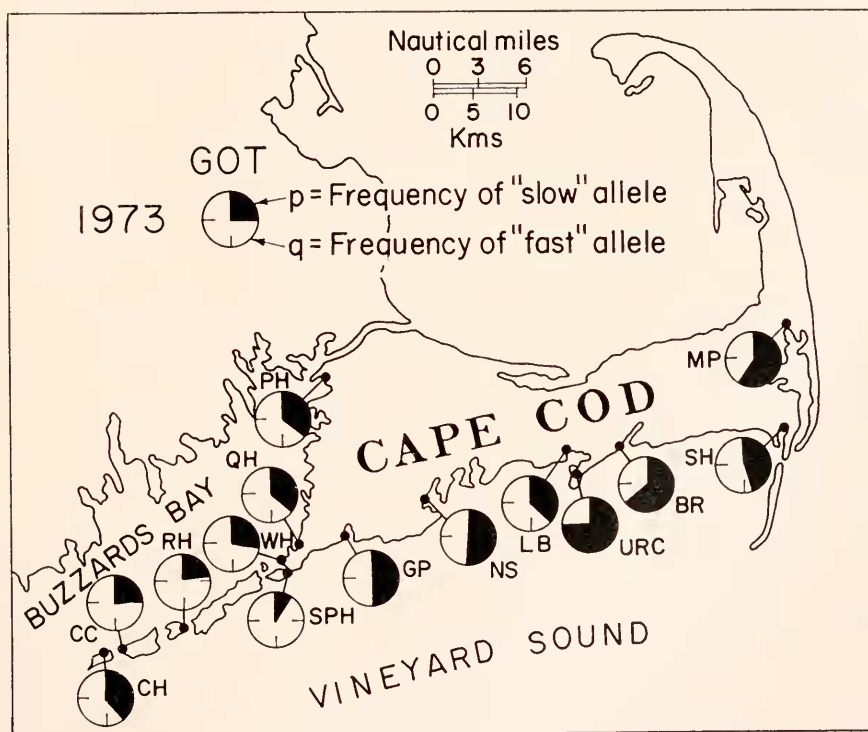

FIGURE 4. Map of allele frequencies (1973) at the polymorphic glutamate oxalate transaminase (GOT) locus. "Fast" and "slow" refer to mobilities of the enzymes on polyacrylamide gels. Note the generally higher frequency of the "fast" allele to the southwest. WH combines WH-1 and WH-2 shallow localities. Localities are listed in Table III.

these enzymes produced by different alleles in ectoprocts. Such data could be used to test the ecologic interpretations presented in this paper.

Since LAP and GOT values at every specific locality are not precisely correlated with each other (see Fig. 5), the differences may be due to selection being somewhat different in the two cases, or to the several types of possible background "noise". Perhaps the species composition of the phytoplankton food of *Schizoporella errata,* which in general varies with water temperature, and yet may differ from
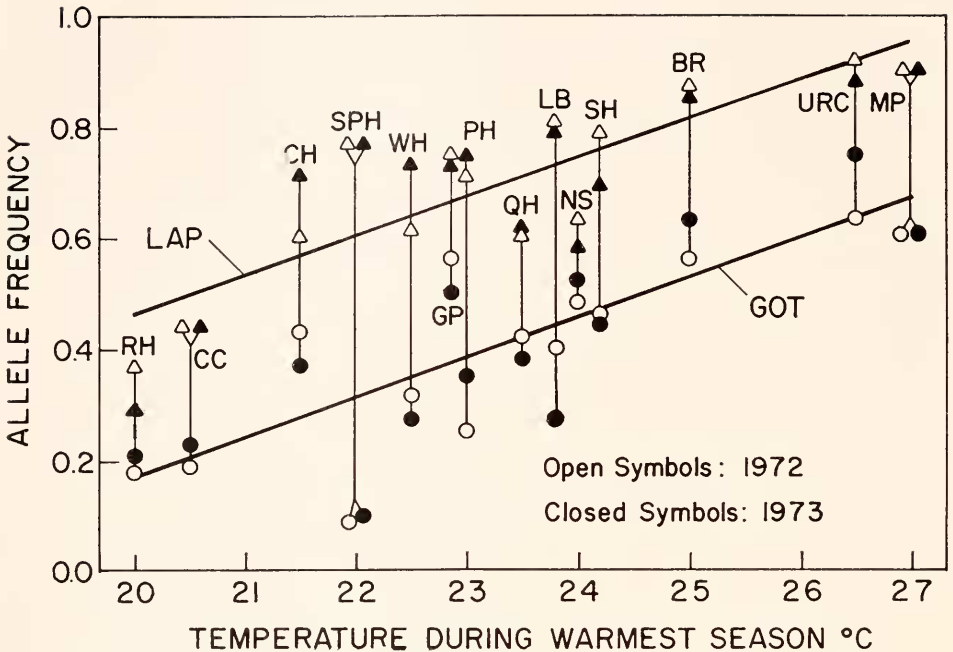
FIGURE 5. Chart of allele frequencies *versus* temperature during warmest season in °C. For each year, points for the two loci (LAP = leucine amino peptidase; GOT = glutamate oxalate transaminase) are from the same organisms collected at the same time, and must necessarily have the same temperature history. If y = allele frequency, and x = temperature, for LAP, $y = -.93 + 0.069x$; for GOT, $y = -1.25 + 0.071x$. Note that the slope of the lines are nearly identical.

place to place, would be especially important by providing differing organic compounds on which the enzymes in question would be acting.

Allele frequencies from successive years are rather close to each other (Tables I–III; Fig. 5). This supports the view that local populations are in some sort of local equilibrium with local ecologic conditions. Fitness surfaces for 1972 and

TABLE IV

Allele frequencies for localities with data for 3 to 5 years. Abbreviations are: n = number of individuals; p = frequency of slow mobility allele; and q = frequency of fast mobility allele

Locality	1969			1970			1971			1972			1973		
	n	p	q	n	p	q	n	p	q	n	p	q	n	p	q
Cuttyhunk Channel				30	0.35	0.65	31	0.39	0.61	27	0.44	0.56	24	0.44	0.56
Robinsons Hole				36	0.31	0.69				24	0.37	0.63	24	0.29	0.71
Sheep Pen Harbor				29	0.62	0.38				47	0.77	0.23	24	0.77	0.23
Woods Hole (VERRILL Dock)	50	0.72	0.28	45	0.61	0.39	58	0.58	0.42	59	0.60	0.40	24	0.62	0.38
Green Pond	43	0.76	0.24	47	0.76	0.24				24	0.75	0.25	24	0.73	0.27
Quissett Harbor							48	0.62	0.38	24	0.60	0.40	24	0.62	0.38
Phinneys Harbor							48	0.80	0.20	24	0.71	0.29	24	0.75	0.25

1973 data have been prepared for warm water, intermediate temperature and cool water localities (Schopf, 1974). These show that there is a change in fitness values for a given genotype in different environments. The importance of selection of different genotypes in different environments was analyzed by Smith (1970) who showed that "a stable polymorphism in a varied environment does not require that the heterozygote is the fittest genotype in any environment, and is in fact possible if one allele is dominant in all environments."

Summary

Gene and genotype frequencies are reported for two polymorphic loci which make the enzymes leucine amino peptidase and glutamate oxalate transaminase for a ubiquitous coastal zone species, the marine ectoproct *Schizoporella errata* (*S. unicornis* of most literature). Allele frequencies were determined from 14 localities in the Cape Cod region for 1972 and repeated in 1973. Gene frequencies for both loci change in a regular manner that is correlated with local water temperature during the warmest season. Allele frequencies have remained fairly uniform in local populations for as long as 5 successive years.

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